L6 ANSWER 11 OF 139 USPATFULL
ACCESSION NUMBER: 1998:64733 USPATFULL
TITLE: Methods and compositions for the early detection and treatment of insulin dependent diabetes mellitus
INVENTOR(S): Atkinson, Mark A., Gainesville, FL, United States
Maclaren, Noel K., Archer, FL, United States
PATENT ASSIGNEE(S): University of Florida Research Foundation, Inc., Gainesville, FL, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5762937	19980609
APPLICATION INFO.:	US 1994-219816	19940328 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1993-93821, filed on 19 Jul 1993, now abandoned which is a continuation of Ser. No. US 1993-7406, filed on 22 Jan 1993, now abandoned which is a continuation of Ser. No. US 1990-569324, filed on 17 Aug 1990, now abandoned which is a continuation-in-part of Ser. No. US 1989-427051, filed on 25 Oct 1989 which is a continuation-in-part of Ser. No. US 1988-283633, filed on 12 Dec 1988, now abandoned
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Cunningham, Thomas M.
LEGAL REPRESENTATIVE: Saliwanchik, Lloyd & Saliwanchik
NUMBER OF CLAIMS: 2
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 1 Drawing Figure(s); 4 Drawing Page(s)
LINE COUNT: 1597
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention pertains to the discovery of antigenic cross reactivity between a pancreatic 64K autoantigen and glutamic acid decarboxylase (GAD). Autoantibodies toward GAD were found to be associated with insulin dependent diabetes (IDD). More specifically, the invention pertains to the use of GAD for the diagnosis, prevention and treatment of insulin dependent diabetes (IDD).

L6 ANSWER 12 OF 139 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1999:13114 BIOSIS
DOCUMENT NUMBER: PREV199900013114
TITLE: Identification and characterization of a novel member of the fibroblast growth factor family.
AUTHOR(S): Greene, J. M.; Li, Y. L.; Yourey, P. A.; Gruber, J.; Carter, K. C.; Shell, B. K.; Dillon, P. A.; Florence, C.; Duan, D. R.; Blunt, A.; Ornitz, D. M.; Ruben, S. M.; Alderson, R. F. (1)
CORPORATE SOURCE: (1) Human Genome Sci. Inc., 9410 Key West Ave., Rockville, MD 20850 USA
SOURCE: European Journal of Neuroscience, (May, 1998) Vol. 10, No. 5, pp. 1911-1925.
ISSN: 0953-816X.
DOCUMENT TYPE: Article
LANGUAGE: English
AB A new member of the fibroblast growth factor (FGF) family, FGF-13, has been molecularly cloned as a result of high throughput sequencing of a **human** ovarian cancer cell library. The open reading frame of the novel **human** gene (1419 bp) encodes for a protein of 216 a.a.

with a molecular weight of 22 kDa. The FGF-13 **sequence** contains an amino-terminal hydrophobic region of 23 a.a. characteristic of a signal secretion **sequence**. FGF-13 is most homologous, 70% similarity at the amino acid level, to FGF-8. Northern hybridization analysis demonstrated prominent expression of FGF-13 in **human** foetal and adult brain, particularly in the cerebellum and cortex. In proliferation studies with BaF3 cells, FGF-13 preferentially activates cell clones expressing either FGF receptor variant, 3-IIIc or 4. The signal transduction pathways of FGF-13 and FGF-2 were compared in rat hippocampal astrocytes. The two FGFs induce an equivalent level of tyrosine phosphorylation of mitogen activated protein kinase (MAPK) and c-raf activation. However, FGF-13 is more effective than FGF-2 in inducing the phosphorylation of phospholipase C-gamma (PLC-gamma). Treatment of neuronal cultures from rat embryonic cortex with FGF-13 increases the number of **glutamic acid decarboxylase** immunopositive neurons, the level of high-affinity gamma-aminobutyric acid (GABA) uptake, and choline acetyltransferase enzyme activity. The GABAergic neuronal response to FGF-13 treatment is rapid with a significant increase occurring within 72 h. We have identified a novel member of the FGF family that is expressed in the central nervous system (CNS) and increases the number as well as the level of phenotypic differentiation of cortical neurons in vitro.

L6 ANSWER 13 OF 139 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1998:347898 BIOSIS
DOCUMENT NUMBER: PREV199800347898
TITLE: Identification of mimicry peptides based on sequential motifs of epitopes derived from 65-kDa glutamic acid decarboxylase.
AUTHOR(S): Bach, Jean-Marie; Otto, Heike; Jung, Guenther; Cohen, Helene; Boitard, Christian; Bach, Jean-Francois; Van Endert, Peter M. (1)
CORPORATE SOURCE: (1) INSERM U25, 161 rue de Sevres, F-75743 Paris Cedex 15 France
SOURCE: European Journal of Immunology, (June, 1998) Vol. 28, No. 6, pp. 1902-1910.
ISSN: 0014-2980.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Insulin-dependent diabetes mellitus (IDDM) is an autoimmune disease with a predominantly non-hereditary etiology that results in a destruction of pancreatic beta cells by autoaggressive T lymphocytes. Neither the mechanism of initial stimulation of these T cells nor the nature of the environmental factors implicated in the disease have so far been identified. However, both issues are taken into account by the hypothesis of initial T cell activation by viral or bacterial mimicry peptides with **sequence** similarities to pancreatic self antigens. We determined sequential epitope motifs to search for mimicry peptides stimulating T cell lines specific for two epitopes derived from the IDDM autoantigen 65-kDa **glutamic acid decarboxylase** (GAD65). These were GAD65 (88-99), presented by HLA-DRB1*0101, and GAD65 (248-257), presented by HLA-DR65*0101. T cell stimulation by peptides with substitutions in HLA anchor or T cell contact positions was analyzed to establish degenerate epitope motifs for database searching. Out of 28 tested candidate mimicry peptides derived from bacterial, viral and **human** proteins, 3 stimulated T cell lines and a T cell clone specific for epitope GAD65 (248-257). Our results demonstrate that mono- and polyclonal GAD65-specific T cells from IDDM patients can be stimulated by viral and bacterial peptides with little apparent **sequence** homology with autoantigenic epitopes. Moreover, in a synopsis with related

published studies, our findings suggest that simple degenerate search motifs comprising principal T cell contacts plus HLA class II binding motifs may suffice to identify most mimicry peptides.

L6 ANSWER 14 OF 139 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1999:35993 BIOSIS
DOCUMENT NUMBER: PREV199900035993
TITLE: Inverse polymerase chain reaction mediated chromosome walking within the **human** glutamic acid decarboxylase gene.
AUTHOR(S): Khairatkar-Joshi, Neelima; Ahmad, Nasir S. (1)
CORPORATE SOURCE: (1) Dep. Nat. Prod. Biol., Res. Cent., Hoechst Marion Roussel Ltd., LBS Road, Mulund, Mumbai-400 080 India
SOURCE: Journal of Biosciences (Bangalore), (Sept., 1998) Vol. 23, No. 3, pp. 265-269.
ISSN: 0250-5991.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Using inverse polymerase chain reaction (PCR), we have cloned partial intronic sequences from **human glutamic acid decarboxylase** (GAD) gene. A small 153 bp core region was selected from the GAD cDNA **sequence** to design outward primers corresponding to its 3' and 5' ends. EcoRI digested **human** DNA which had been circularized by self-ligation and then linearized with SacII was used as a substrate to carry out PCR. This gave a 900 bp long product which was cloned into pUC 19. The **sequence** analysis of this fragment revealed the presence of introns in the region flanking the selected core DNA. In this work we used this technique to walk into the upsteam region of the GAD gene using **sequence** information from its cloned cDNA.

L6 ANSWER 15 OF 139 (c) 1999 FAO (on behalf of the ASFA Advisory Board) All rights reserved.
ACCESSION NUMBER: 1998:41340 AQUASCI
DOCUMENT NUMBER: ASFA1 1998
TITLE: **Sequence** and expression of **glutamic acid decarboxylase** isoforms in the developing zebrafish
AUTHOR: Martin, S.C.; Heinrich, G.; Sandell, J.H.*
CORPORATE SOURCE: Department of Anatomy and Neurobiology, Boston University School of Medicine, 80 East Concord Street, R1014, Boston, MA 02118, USA
SOURCE: Journal of Comparative Neurology, (19980629) vol. 396, no. 2, pp. 253-266.
ISSN: 0021-9967.
DOCUMENT TYPE: Journal
FILE SEGMENT: ASFA1
LANGUAGE: English
SUMMARY LANGUAGE: English
AB We describe the isolation two glutamic acid decarboxylase (GAD) cDNAs from zebrafish with over 84% identity to **human** GAD65 and GAD67. In situ hybridization studies revealed that both GAD65 and GAD67 were expressed in the early zebrafish embryo during the period of axonogenesis, suggesting a role for GABA prior to synapse formation. Both GAD genes were detected in the telencephalon, in the nucleus of the medial longitudinal fasciculus in the midbrain, and at the border regions of the rhombomeres in the rostral hindbrain. In the caudal hindbrain, only GAD67 was detected (in neurons with large-caliber axons). In the spinal cord, both GAD genes were detected in dorsal longitudinal neurons, commissural secondary ascending neurons, ventral longitudinal neurons, and Kolmer-Agduhr neurons. Immunohistochemistry for gamma -aminobutyric acid (GABA) revealed that GABA is produced at all sites of GAD expression, including

the novel cells in the caudal hindbrain. These results are discussed in the context of the hindbrain circuitry that supports the escape response. We conclude that fish, like mammals, have two GAD genes. The zebrafish GAD65 and GAD67 are present in identified neurons in the forebrain, midbrain, hindbrain, and spinal cord, and they catalyze the production of GABA in the developing embryo.

L6 ANSWER 16 OF 139 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1998:296710 BIOSIS
DOCUMENT NUMBER: PREV199800296710
TITLE: T-cell epitopes in type 1 diabetes autoantigen tyrosine phosphatase IA-2: Potential for mimicry with rotavirus and other environmental agents.
AUTHOR(S): Honeyman, Margo C. (1); Stone, Natalie L.; Harrison, Leonard C. (1)
CORPORATE SOURCE: (1) Walter Eliza Hall Inst. Med. Res., Royal Melbourne Hosp., Victoria 3050 Australia
SOURCE: Molecular Medicine (New York), (April, 1998) Vol. 4, No. 4,
pp. 231-239.
ISSN: 1076-1551.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The tyrosine phosphatase IA-2 is a molecular target of pancreatic islet autoimmunity in type 1 diabetes. T-cell epitope peptides in autoantigens have potential diagnostic and therapeutic applications, and they may hold dues to environmental agents with similar sequences that could trigger or exacerbate autoimmune disease. We identified 13 epitope peptides in IA-2 by measuring peripheral blood T-cell proliferation to 68 overlapping, synthetic peptides encompassing the intracytoplasmic domain of IA-2 in six at-risk type 1 diabetes relatives selected for HLA susceptibility haplotypes. The dominant epitope, VIVMLTPLVEDGVKQC (aa 805820), which elicited the highest T-cell responses in all at-risk relatives, has 56% identity and 100% similarity over 9 amino adds (aa) with a **sequence** in VP7, a major immunogenic protein of **human** rotavirus. Both peptides bind to HLA-DR4(*0401) and are deduced to present identical aa to the T-cell receptor. The contiguous **sequence** of VP7 has 75% identity and 92% similarity over 12 aa with a known T-cell epitope in **glutamic acid decarboxylase** (GAD), another autoantigen in type 1 diabetes. This dominant IA-2 epitope peptide also has 75-45% identity and 88-64% similarity over 8-14 aa to sequences in Dengue, cytomegalovirus, measles, hepatitis C, and canine distemper viruses, and the bacterium Haemophilus influenzae. Three other IA-2 epitope peptides are 71-100% similar over 7-12 aa to herpes, rhino-, hanta- and flaviviruses. Two others are 80-82% similar over 10-11 aa to sequences in milk, wheat, and bean proteins. Further studies should now be carried out to directly test the hypothesis that T-cell activation by rotavirus and possibly other viruses, and dietary proteins, could trigger or exacerbate beta-cell autoimmunity through molecular mimicry with IA-2 and (for rotavirus) GAD.

L6 ANSWER 17 OF 139 AIDSLINE
ACCESSION NUMBER: 1999:3610 AIDSLINE
DOCUMENT NUMBER: MED-99054184
TITLE: Peptide from glutamic acid decarboxylase similar to coxsackie B virus stimulates IFN-gamma mRNA expression in Th1-like lymphocytes from children with recent-onset insulin-dependent diabetes mellitus.
AUTHOR: Karlsson M G; Ludvigsson J
CORPORATE SOURCE: Department of Health and Environment, Faculty of Health Sciences, Linkoping University, Sweden.
SOURCE: ACTA DIABETOLOGICA, (1998). Vol. 35, No. 3, pp. 137-44.
Journal code: A80. ISSN: 0940-5429.

PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
FILE SEGMENT: MED; Priority Journals
LANGUAGE: English
OTHER SOURCE: MEDLINE 99054184
ENTRY MONTH: 199904

AB At the clinical onset of insulin-dependent diabetes mellitus (type 1 diabetes), inflammation within the pancreatic islets of Langerhans causes insulitis. CD4+ or Th-lymphocytes will be activated after stimulation resulting in interferon-gamma (IFN-gamma) production by Th1-like lymphocytes and/or interleukin-4 (IL-4) secretion from Th2-like lymphocytes. The antigens responsible for this activation are unknown, but studies have suggested **glutamic acid decarboxylase** (GAD) to be a possible candidate. One peptide from this enzyme (amino acid 247-279) with a similar amino acid **sequence** to coxsackie B virus may cause lymphocyte proliferation in diabetic patients. In this study we have shown that this peptide activates Th1-like lymphocytes which produce increased amounts of IFN-gamma mRNA, but seldom mRNA for IL-4. Lymphocytes from healthy HLA-matched controls (DR3/4) did not respond with an upregulated mRNA expression for these cytokines when stimulated by the GAD-peptide ($P<0.05$). A low or absent expression of IFN-gamma mRNA was significantly correlated to a high fasting C-peptide at 3 months' duration ($P<0.05$). In conclusion, we suggest that GAD65 is involved in the development of type 1 diabetes and that the Th1-response may play a role in the destruction of beta cells.

L6 ANSWER 18 OF 139 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1998:405106 BIOSIS
DOCUMENT NUMBER: PREV199800405106
TITLE: Peptide specificity of high-titer anti-glutamic acid decarboxylase (GAD)65 autoantibodies.
AUTHOR(S): Rharbaoui, Faiza (1); Granier, Claude; Kellou, Mouna; Mani, Jean-Claude; Van Endert, Peter; Madec, Anne-Marie; Boitard, Christian; Pau, Bernard; Bouanani, Majida
CORPORATE SOURCE: (1) CNRS-UMR9921, Faculte de Pharmacie, 15 avenue Charles Flahault, 34060 Montpellier Cedex 2 France
SOURCE: Immunology Letters, (July, 1998) Vol. 62, No. 3, pp. 123-130.
ISSN: 0165-2478.
DOCUMENT TYPE: Article
LANGUAGE: English

AB To study systematically the linear epitope specificity of anti-**glutamic acid decarboxylase** (GAD) autoantibodies associated with insulin-dependent diabetes mellitus (IDDM), we produced 93 overlapping 12-residue synthetic peptides derived from the **sequence** of the **human** GAD65 protein and covering the entire length of the protein. These peptides were used as antigens in an enzyme immunoassay to screen the sera from 10 IDDM patients, all of which contained at high level autoantibodies directed against GAD65. Three out of ten (30%) IDDM patients had antibodies that reacted with one or more of the synthetic peptides. Two of the peptide-reactive IDDM sera, which also bound denatured recombinant GAD65 on western blots, had the highest titers of anti-GAD antibodies in ELISA assay. Moreover, the anti-GAD antibodies-GAD complexes formed with these sera were characterized by low dissociation rates, indicative of their good stability. A fine specificity analysis, using analogs of antigen peptide 1 (residues 1-12), allowed us to identify the residues at positions 5-9 (GSGFW) as critical for antibody

recognition.

L6 ANSWER 19 OF 139 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1998:164066 BIOSIS
DOCUMENT NUMBER: PREV199800164066
TITLE: Molecular mimicry in diabetes mellitus: The homologous domain in coxsackie B virus protein.
AUTHOR(S): Vreugdenhil, G. R. (1); Geluk, A.; Ottenhoff, T. H. M.; Melchers, W. J. G.; Roep, B. P.; Galama, J. M. D.
CORPORATE SOURCE: (1) Univ. Nijmegen, Dep. Med. Microbiol., P.O. Box 9101, NL-6500 HB Nijmegen Netherlands
SOURCE: Diabetologia, (Jan., 1998) Vol. 41, No. 1, pp. 40-46. ISSN: 0012-186X.
DOCUMENT TYPE: Article
LANGUAGE: English
AB It has been proposed that molecular mimicry between protein 2C (p2C) of coxsackie virus B4 and the autoantigen **glutamic acid decarboxylase** (GAD65) plays a role in the pathogenesis of insulin-dependent diabetes mellitus (IDDM). In this study we show that the amino acid **sequence** of p2C which shares homology with a **sequence** in GAD65 (PEVKEK), is highly conserved in coxsackie virus B4 isolates as well as in different viruses of the subgroup of coxsackie B-like enteroviruses. These are the most prevalent enteroviruses and therefore exposure to the mimicry motif will be a frequent event throughout life. Presentation of the homologous peptides by HLA molecules is essential for T-cell reactivity. Therefore, we tested whether the PEVKEK motif can bind to the IDDM-associated HLA-DR1, -DR3 and -DR4 molecules. Synthetic peptides with sequences derived from p2C and GAD65 did bind to HLA-DR3 but not to HLA-DR1 or -DR4. Replacement of amino acids within the motif showed that the PEVKEK motif binds specifically to HLA-DR3. Moreover, both p2C and GAD65 peptides bind in the same position within the peptide binding groove of the DR3 molecule which is an essential requirement for T-cell cross-reactivity The results support molecular mimicry between p2C of coxsackie B-like enteroviruses and GAD65. However, this molecular mimicry may be limited to the HLA-DR3 positive sub-population of IDDM patients.

L6 ANSWER 20 OF 139 AIDSLINE
ACCESSION NUMBER: 1999:376 AIDSLINE
DOCUMENT NUMBER: MED-98364243
TITLE: Determination of mRNA expression for IFN-gamma and IL-4 in lymphocytes from children with IDDM by RT-PCR technique.
AUTHOR: Karlsson M G; Ludvigsson J
CORPORATE SOURCE: Department of Health and Environment, Faculty of Health Sciences, University Hospital, Linkoping, Sweden. maria.karlsson@kfc.liu.se
SOURCE: DIABETES RESEARCH AND CLINICAL PRACTICE, (1998). Vol. 40, No. 1, pp. 21-30.
Journal code: EBI. ISSN: 0168-8227.
PUB. COUNTRY: Ireland
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
FILE SEGMENT: MED; Priority Journals
LANGUAGE: English
OTHER SOURCE: MEDLINE 98364243
ENTRY MONTH: 199901
AB Insulin-dependent diabetes mellitus (IDDM) is characterized by infiltration of T-lymphocytes in the islets of Langerhans. Antigens are presented to Th-lymphocytes which can be divided into Th1- and Th2-lymphocytes, producing interferon-gamma (IFN-gamma) and interleukin-4 (IL-4) respectively. The aim of our study was to determine the messenger-RNA (mRNA) for these cytokines by RT-PCR in antigen-stimulated lymphocytes from children with newly diagnosed IDDM. The expression of mRNA for IL-4, and to a lesser degree IFN-gamma, is increased in

lymphocytes stimulated with tetanus toxoid (TT). Loss of activity after freezing and thawing could be compensated for, by increased amplification, while the use of EDTA or sodium heparin in the blood samples did not influence the results. In a pilot application, the lymphocytes from children with newly diagnosed IDDM were stimulated with a peptide of **glutamic acid decarboxylase** (GAD) (a.a. 247-279) known to have a similar aminoacid **sequence** as the Coxsackie B virus (a.a. 32-47). Increased IFN-gamma mRNA could be seen in two out of four children, whereas IL-4 showed a less pronounced mRNA expression. No increased mRNA expression for IFN-gamma and IL-4 could be seen in healthy HLA-matched controls. Further studies are needed to confirm whether increased IFN-gamma mRNA in Th1-like lymphocytes stimulated with this specific GAD-peptide play a role in the cell-mediated immune response seen in children early after the onset of IDDM.

L6 ANSWER 21 OF 139 BIOTECHDS COPYRIGHT 1999 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1997-12649 BIOTECHDS
TITLE: Myeloma cell line expressing recombinant **human glutamic-acid-decarboxylase**; recombinant autoantigen expression in SPG14 cell culture, for use in insulin-dependent diabetes mellitus diagnosis
AUTHOR: Matsuba T; Yasukawa K
PATENT ASSIGNEE: Tosoh
LOCATION: Yamaguchi, Japan.
PATENT INFO: EP 798379 1 Oct 1997
APPLICATION INFO: EP 1997-302110 26 Mar 1997
PRIORITY INFO: JP 1996-76681 29 Mar 1996
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1997-473189 [44]

AB A new myeloma cell line (e.g. SPG14) expresses a **human** recombinant glutamate-decarboxylase (EC-4.1.1.15) gene. The recombinant enzyme product may be purified and used in an immunoassay to detect anti-**human** glutamate-decarboxylase antibodies, or may be used to immunize an animal for production of antibodies. Glutamate-decarboxylase is an autoantigen linked with insulin-dependent diabetes mellitus, and may be useful in diabetes diagnosis. The enzyme may also be used to investigate the steric structure of the enzyme, and bonding with autoantibodies. Enzyme-specific B-lymphocytes and T-lymphocytes may be used for adoptive immunotherapy of diabetes. Since glutamate-decarboxylase productivity of e.g. pig brain is low, production of the enzyme by recombinant methods allows recovery of mg quantities, which allows use of ELISA rather than RIA. In an example, the **human** GAD65 gene was amplified from a **human** pancreas cDNA library, and a 1.8-kb fragment was cloned in plasmid pBluescript and plasmid BCMGS-GAD, for expression in an Sp2/0 cell culture. (16pp)